

# Mate choice and imprinting in birds studied by cross-fostering in the wild

Tore Slagsvold<sup>1\*</sup>, Bo T. Hansen<sup>1</sup>, Lars E. Johannessen<sup>1</sup> and Jan T. Lifjeld<sup>2</sup>

<sup>1</sup>Department of Biology, University of Oslo, PO Box 1050, Blindern, N-0316 Oslo, Norway  
(tore.slagsvold@bio.uio.no, b.t.hansen@bio.uio.no, l.e.johannessen@bio.uio.no)

<sup>2</sup>Zoological Museum, University of Oslo, PO Box 1172, Blindern, N-0318 Oslo, Norway (j.t.lifjeld@nhm.uio.no)

Sexual-selection theories generally assume that mating preferences are heritable traits. However, there is substantial evidence that the rearing environment may be important for the development of mating preferences, indicating that they may be learnt, or modified by experience. The relative importance of such sexual imprinting across species remains largely unexplored. Here, we report results of a large-scale cross-fostering experiment in the wild in which nestling birds were raised by parents of a different species. We show that resulting sexual imprinting may have a negative effect on pairing success in one species (the great tit, *Parus major*), but not in two other species (the blue tit, *P. caeruleus* and the pied flycatcher, *Ficedula hypoleuca*). A remarkable variation thus seems to exist, even between species that are congeneric and have similar breeding ecologies. The cross-fostering resulted in heterospecific pairings between the two tit species (female blue tit breeding with male great tit), which has never, to our knowledge, been previously documented. However, the chicks fledging from these nests were all blue tit.

**Keywords:** cross-fostering; hybridization; learning; mate choice; pairing success; sexual imprinting

## 1. INTRODUCTION

Many animals exhibit strong mating preferences and sexual selection theories generally assume that such preferences are heritable traits (Andersson 1994; Jennions & Petrie 1997). Much research has taken place to document their genetic basis, for example through selection experiments or estimates of additive genetic variance components (Bakker & Pomiankowski 1995; Pomiankowski & Møller 1995; Jennions & Petrie 1997). However, to understand the evolutionary role of mating preferences we also need to know how they are shaped by other factors, like social learning. Learning from parents during early development may crucially influence future mate-choice decisions of young birds (ten Cate & Vos 1999) and mammals (Kendrick *et al.* 1998; Penn & Potts 1998). Recently, the importance of this well-known process of sexual imprinting has been recognized in the fields of sexual selection, hybridization, speciation and brood parasitism (Laland 1994; Grant & Grant 1997; Price 1998; Irwin & Price 1999; Owens *et al.* 1999; ten Cate & Vos 1999; Witte *et al.* 2000; Slagsvold & Hansen 2001). In birds, sexual imprinting seems to be the rule rather than the exception (ten Cate & Vos 1999), but little is known about the relative importance of learning and genes in shaping the sexual preferences and how this may vary among species.

Sexual imprinting is supposed to occur in two stages; an early acquisition phase where a sexual preference is established and a subsequent consolidation stage where the early-acquired preference is linked to sexual behaviour and stabilized (Immelmann *et al.* 1991; Kruijt & Meeuwissen 1991; Oetting *et al.* 1995; Oetting & Bischof 1996). This means that the initial sexual imprinting can be modified

from social experience (ten Cate 1984; Immelmann *et al.* 1991; Kruijt & Meeuwissen 1991; Oetting & Bischof 1996), as reflected in physiological changes in specific areas of the forebrain (Bischof & Rollenhagen 1999; Rollenhagen & Bischof 2000). However, the results were mainly obtained from experiments in captivity with isolated birds. The hypotheses should therefore be tested under more natural circumstances (Kruijt & Meeuwissen 1991; Oetting *et al.* 1995; Oetting & Bischof 1996).

We have studied sexual imprinting in the wild by cross-fostering passerine birds that differ in relatedness, body size and social behaviour. In an initial study, we let great tits *Parus major* be reared by blue tits *P. caeruleus*. The chicks survived well in the nest and afterwards (Slagsvold 1998; Slagsvold & Hansen 2001), but appeared to be strongly sexually mis-imprinted on the blue tit host (Slagsvold & Hansen 2001). In the present study, we conducted a reciprocal experiment by letting great tits be reared by blue tits and blue tits be reared by great tits in the same study area and in the same breeding seasons. The two species are resident and social and live in mixed species flocks outside the breeding season. Most pairs are formed long before breeding commences (Perrins 1979), providing ample opportunity for sexual contact between the two species before breeding. Their breeding ecologies are also similar and hence we expected the effect of cross-fostering to be similar. However, one difference between the two species is that great tits (adult weight *ca.* 17–20 g) are socially dominant to blue tits (adult weight *ca.* 10–11 g). If the initial sexual imprinting can be modified from social experience, we might expect cross-fostered blue tits to become less mis-imprinted than cross-fostered great tits because of the asymmetry in social dominance. For instance, a cross-fostered male blue tit displaying towards an unmanipulated female great tit may be physically defeated by her, which may weaken the initial sexual imprinting. In comparison, a cross-fostered male great tit

\* Author for correspondence.

displaying towards an unmanipulated female blue tit may be met by avoidance, which may have a less modifying effect on the initial sexual imprinting. To test this idea further, we also let blue tits be reared by a smaller, congeneric species, the coal tit *P. ater* (adult weight ca. 9–10 g), which is socially subordinate to blue tits. We asked if these cross-fostered blue tits became more sexually imprinted on their host than blue tits reared by great tits.

In the final experiment, we let pied flycatchers *Ficedula hypoleuca* (adult weight ca. 11–13 g) be reared by great tits and blue tits. Pied flycatchers are migratory and live more solitarily than the tits (Lundberg & Alatalo 1992), which may select for sexual preferences to be more innate (Slagsvold & Hansen 2001). They spend the winter in tropical Africa where the two tits are not found, giving cross-fostered individuals less opportunity for consolidation of the initial sexual imprinting. In addition, cross-fostered pied flycatchers were less closely related to their host species than were the cross-fostered tits. Hence, we hypothesized that sexual imprinting on the foster species would be weak in cross-fostered pied flycatchers.

## 2. MATERIAL AND METHODS

The study was carried out in woodland plots provided with nest boxes near Oslo, Norway, under licence from the Directorate for Nature Management and from the National Animal Research Authority in Norway. Cross-fostering of tits was done in a study area with about 70 breeding pairs of great tits per year, 80 pairs of blue tits and a few pairs of coal tits. Cross-fostering of pied flycatchers was done in three other study plots. Unfortunately, great tit and blue tit chicks will not thrive in pied flycatcher nests (T. Slagsvold, unpublished data), so a reciprocal experiment was not possible, in this case.

Eggs were exchanged during the incubation period and all host eggs were removed. Great tits were reared by blue tits (155 chicks fledging from 41 nests) and blue tits by great tits (242 chicks fledging from 41 nests) in 1999–2000. Blue tits were reared by coal tits in 2000 (38 chicks fledging from six nests) and pied flycatchers were reared by great tits and blue tits in 1998–1999 (573 chicks fledging from 139 nests). Chicks reared in unmanipulated nests in the same study plots and years served as controls (196 great tits fledging from 40 nests, 175 blue tits fledging from 32 nests and 935 pied flycatchers fledging from 173 nests). All birds were ringed for later identification and body mass was recorded to the nearest 0.1 g with a Pesola spring balance when 15 (tits) or 13 days old (flycatchers). Tits were recaptured in their first autumn for measurement of body mass, wing length (with flattened and straightened wing) and tarsus length (with bent toes). A few tits and all adult pied flycatchers were measured when 2 years old. The ratio between body mass and wing length was used as a measure of condition.

Local recruitment was calculated as the proportion of birds observed after 15 March in year  $x + 1$  (or later) of the birds fledging in year  $x$ . A tit was assumed to be paired if it was seen repeatedly with a mate after 15 March. It was considered to be unpaired if it was still present in the area after 1 May and never seen with a mate. These two groups of birds are hereafter termed 'resident'. All other tits were excluded from the analyses of pairing success. This means that unpaired tits disappearing before 1 May were excluded from the analysis because they might have paired outside the study area. A pied flycatcher was considered

to be paired if it was seen repeatedly with a mate and considered as unpaired if repeatedly seen without a mate during a period of at least one week and was never seen with a mate afterwards. Pairing success was only calculated for yearling birds because their response would best reflect the initial sexual imprinting. Data for all recruits were pooled in the analyses because there were few recruits originating from the same broods and because the frequency distributions of the number of recruits per brood did not deviate significantly from random expectations for any of the groups. Data were also pooled across years because no significant annual variation was found in local recruitment or pairing success for any group of birds. Statistical tests were two-tailed. *G*-tests are presented with William's correction. In order to avoid multiple testing of the same hypothesis, a sequential Bonferroni correction was applied, when necessary (Rice 1989). The number of tests corrected for is given by the value of  $k$ . The corrected  $\alpha$ -level is denoted by  $\alpha_{\text{B}}$  (B for Bonferroni) with a numerical subscript indicating the significance level of the particular test.

In six cases, a female blue tit nested with a male great tit. For correct species identification of the offspring of these pairs, we performed a genetic analysis of blood (31 chicks) or tissue samples (two dead chicks), using two microsatellite DNA markers and one randomly amplified polymorphic (RAPD) DNA marker, each of which conclusively separates the two species. The POCC8 microsatellite marker (Bensch *et al.* 1997) is monomorphic (204 bp) in blue tits and dimorphic (203 and 209 bp) in great tits. The PAT MP 2-43 microsatellite marker (Otter *et al.* 1998) shows a non-overlapping allele size distribution in the two species; four alleles of 140 bp or more in blue tits and six alleles 135 bp or less in the great tit. Finally, the microsatellite forward primer, PK12-F (Tanner 1995), when used alone as a RAPD marker, amplifies two clearly resolved, species-specific bands in blue tits and three such bands in great tits. These results were obtained from the analysis of 20 unrelated individuals of each species, using an ABI PRISM TM 310 (Perkin Elmer) automated sequencer for the two microsatellites and agarose gel electrophoresis for the RAPD marker.

## 3. RESULTS

Cross-fostering did not significantly reduce local recruitment to the breeding population in the following year in any of the three species involved (figure 1a; table 1). It was similar for great tits reared by blue tits and great tit controls (18/155 versus 25/196;  $G = 0.11$ ,  $p = 0.75$ ) and for blue tits reared by great tits and blue tit controls (24/242 versus 12/175;  $G = 1.23$ ,  $p = 0.27$ ); higher for blue tits reared by coal tits than blue tit controls (7/38 versus 12/175;  $G = 4.30$ ,  $p = 0.038$ ); and similar for pied flycatchers reared by great tits and blue tits and pied flycatcher controls (25/573 versus 55/935;  $G = 1.67$ ,  $p = 0.20$ , combining all cross-fostered pied flycatchers).

Cross-fostering affected pairing success in great tits, but not in blue tits or in pied flycatchers (figure 1b; table 1). Pairing success was significantly lower for great tits reared by blue tits than for great tit controls (3/11 versus 19/20;  $G = 16.26$ ,  $p = 0.0001$ ,  $\alpha_{\text{B},0.001} = 0.0003$ ,  $k = 4$ ; figure 1b), also when considering birds that engaged in heterospecific pairings as having failed to mate (0/11 versus 19/20;  $p < 0.0001$ ,  $\alpha_{\text{B},0.001} = 0.0003$ ,  $k = 4$ , Fisher exact test; figure 1b). The latter difference also held true in separate analyses for males (0/9 versus 9/10;  $p = 0.0001$ ,

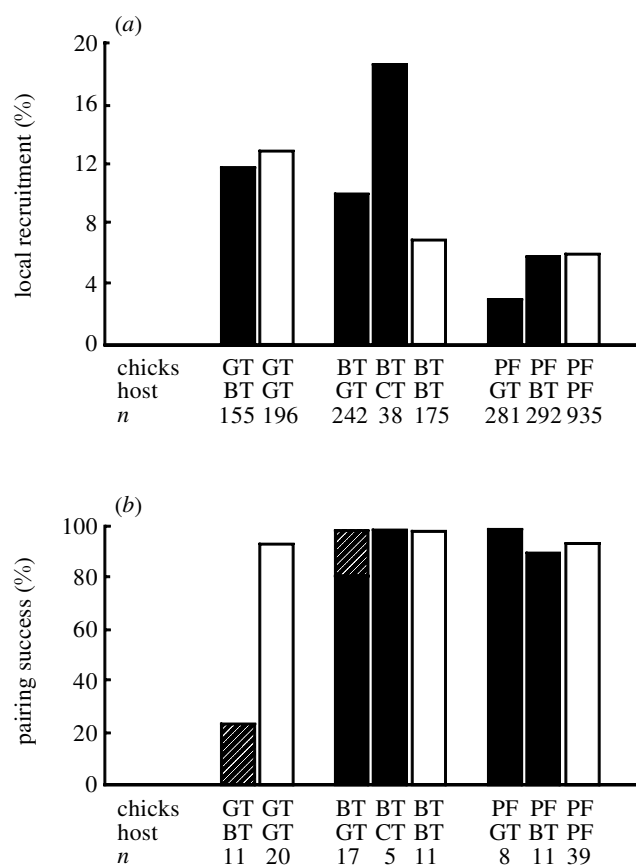


Figure 1. (a) Local recruitment and (b) pairing success of cross-fostered (filled and hatched bars) and control birds (open bars). Cross-fostered great tits (GT) were reared by blue tits (BT), cross-fostered blue tits were reared by great tits or coal tits (CT) and cross-fostered pied flycatchers (PF) were reared by great tits or blue tits. Some cross-fostered great tits and blue tits engaged in heterospecific pairings (hatched bars). *n*, sample size.

$\alpha_{B,0.001} = 0.0005$ ,  $k = 4$ ) and females (0/2 versus 10/10;  $p = 0.015$ ,  $\alpha_{B,0.05} = 0.05$ ,  $k = 4$ ). The two cross-fostered females involved both attempted nesting without any help from a social mate.

In contrast to great tits, cross-fostered blue tits generally chose a conspecific mate (figure 1b; table 1). The pairing success for blue tits reared by great tits was similar to that for blue tit controls (17/17 versus 11/11;  $p = 1.00$ , Fisher exact test; figure 1b), also when considering birds that engaged in heterospecific pairings as having failed (14/17 versus 11/11,  $p = 0.26$ ; figure 1b).

Pairing success for cross-fostered blue tits was much higher than that for cross-fostered great tits (17/17 versus 3/11;  $p < 0.0001$ ,  $\alpha_{B,0.001} = 0.0003$ ,  $k = 3$ , Fisher exact test; figure 1b), also when considering birds that engaged in heterospecific pairings as having failed (14/17 versus 0/11;  $p < 0.0001$ ,  $\alpha_{B,0.001} = 0.0005$ ,  $k = 3$ , figure 1b). Of resident yearling birds, great tits reared by blue tits had fledged from smaller broods ( $x = 4.2$ , s.d. = 0.9,  $n = 10$ ) than blue tits reared by great tits ( $x = 6.8$ , s.d. = 1.2,  $n = 17$ ;  $t = 5.81$ ,  $p < 0.001$ ) and so had fewer conspecific brood mates. However, for cross-fostered birds fledging from broods of the same sizes (four or five chicks), a significant difference still existed between the two species in pairing success with a conspecific bird (0/9 in great tits

versus 2/2 in blue tits;  $p = 0.018$ ,  $\alpha_{B,0.05} = 0.05$ ,  $k = 3$ , Fisher exact test). The pairing success for controls was high for both sexes in both species (great tits: 9/10 in males, 10/10 in females; blue tits: 7/7 in males, 4/4 in females), indicating that the population sex ratios were not skewed, which might otherwise have confounded the comparison of cross-fostered birds.

All blue tits reared by coal tits paired with a conspecific bird (figure 1b) in significant contrast with great tits reared by a smaller species (blue tit), where none paired with a conspecific (5/5 versus 0/11;  $p = 0.0002$ ,  $\alpha_{B,0.001} = 0.0005$ ,  $k = 2$ , Fisher exact test). The pairing success of blue tits reared by coal tits was therefore similar to that of blue tits reared by great tits (5/5 versus 17/17;  $p = 1.00$ ). The pairing success was also high for cross-fostered pied flycatchers and similar to that for controls (18/19 versus 37/39;  $G = 0.00$ ,  $p = 0.98$ ; figure 1b). There were no tits present that had been cross-fostered to pied flycatchers, reducing the likelihood of heterospecific pairs between tits and flycatchers. However, the difference in pairing success between cross-fostered great tits and pied flycatchers (3/11 versus 18/19;  $G = 15.66$ ,  $p = 0.0001$ ,  $\alpha_{B,0.001} = 0.001$ ,  $k = 2$ ) held true even when we excluded the cases with heterospecific pairings between great tits and blue tits (0/8 versus 18/19;  $p < 0.0001$ ,  $\alpha_{B,0.001} = 0.0005$ ,  $k = 2$ , Fisher exact test).

A similar number of great tits reared by blue tits and blue tits reared by great tits paired heterospecifically (3/11 versus 3/17;  $G = 0.36$ ,  $p = 0.55$ ). The three blue tits involved in such pairings, all cross-fostered females, paired with cross-fostered great tit males (table 1). In the subsequent year, one of these females again paired with a cross-fostered great tit (a different male), though a male blue tit was also present feeding the young at this nest. Another of the female blue tits that was involved in a heterospecific pairing as a yearling paired with a blue tit when 2 years old. Furthermore, two cross-fostered blue tit females that had nested with unmanipulated blue tits as yearlings paired with cross-fostered great tits when 2 years old.

DNA analyses of the offspring of the six heterospecific pairs (female blue tit breeding with male great tit), using three independent markers, revealed consistently that all 33 offspring were blue tit. Five of the six nests produced fledged young ( $n = 31$ ). In the sixth case, the chicks died soon after hatching in a period of bad weather and tissue samples were only obtained from two chicks. The results show that all of the cross-fostered female blue tits paired with great tits had copulated with male blue tits.

We investigated whether cross-fostered birds present as yearlings differed in quality from conspecific yearling controls, comparing aspects of the rearing conditions (hatching date, nestling body mass) and size and morphology at the first capture after independence. For great tits and blue tits (table 2), none of the differences between the two groups was statistically significant (separate *t*-tests for each sex,  $p > 0.05$ ). This also held true when combining the data for the two sexes and applying ANOVA with sex as a factor when this variable was significant. Similar analyses were also made for the pied flycatcher (data not shown) and no significant differences were found between the treatment groups, except that cross-fostered birds had

Table 1. Local recruitment and pairing success for males (M) and females (F) of cross-fostered birds and of controls.

species	host	chicks initially ringed	observed after 15th March <sup>a</sup>		resident in study area as yearling		paired with conspecific as yearling		paired with heterospecific as yearling		unpaired as yearling	
			M	F	M	F	M	F	M	F	M	F
great tit	blue tit	155	11	7	9	2	0	0	3	0	6	2
great tit	great tit	196	12	13	10	10	9	10	0	0	1	0
blue tit	great tit	242	11	13	7	10	7	7	0	3	0	0
blue tit	coal tit	38	6	1	4	1	4	1	0	0	0	0
blue tit	blue tit	175	8	4	7	4	7	4	0	0	0	0
pied flycatcher	great tit	281	3	5	3	5	3	5	0	0	0	0
pied flycatcher	blue tit	292	9	8	5	6	4	6	0	0	1	0
pied flycatcher	pied flycatcher	935	32	23	24	15	22	15	0	0	2	0

<sup>a</sup> Second year of life or later.

Table 2. Characteristics of cross-fostered and control tits resident in the study area as yearlings.

species	variable	males						females					
		cross-fostered			control			cross-fostered			control		
		mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n
great tit	hatching date <sup>c</sup>	27.6	2.5	8	32.3	10.0	10	23.0	1.4	2	27.5	9.1	10
	body mass (g)	18.0	0.6	8	17.1	1.7	10	17.7	1.2	2	17.2	1.4	10
	15 days old												
	body mass (g) <sup>a</sup>	17.9	0.3	7	18.9	1.2	10	16.5	0.0	2	18.1	1.3	9
	wing length (mm) <sup>a</sup>	76.4	0.6	8	76.0	1.7	10	72.5	3.5	2	72.9	2.0	9
	body mass × 100/wing length <sup>a</sup>	23.4	0.5	7	24.8	1.8	10	22.8	1.1	2	24.8	1.6	9
blue tit <sup>b</sup>	tarsus length (mm) <sup>a</sup>	22.9	0.3	8	22.6	0.3	10	22.2	0.5	2	21.7	0.9	9
	hatching date <sup>c</sup>	24.4	5.0	11	26.4	5.7	7	27.1	5.4	11	30.7	4.2	3
	body mass (g)	11.7	0.6	11	11.4	1.2	7	11.3	0.7	11	11.5	0.0	2
	15 days old												
	body mass (g) <sup>a</sup>	11.4	5.0	11	11.7	0.8	7	11.0	0.7	10	10.8	0.5	3
	wing length (mm) <sup>a</sup>	67.4	1.6	11	67.1	0.9	7	65.4	1.5	10	64.7	1.5	3
	body mass × 100/wing length <sup>a</sup>	16.9	0.9	11	17.4	1.2	7	16.8	1.0	10	16.7	1.1	3
	tarsus length (mm) <sup>a</sup>	19.8	0.3	11	19.6	0.4	7	19.1	0.4	9	18.9	1.0	3

<sup>a</sup> First autumn of life or later.

<sup>b</sup> Cross-fostered birds were reared by great tits or coal tits.

<sup>c</sup> 1 indicates 1 May, etc.

hatched earlier than controls ( $t = 4.77$ , d.f. = 57,  $p = 0.0001$ ,  $\alpha_{B,0.001} = 0.0002$ ,  $k = 6$ ).

4. DISCUSSION

This study suggests that the impact of sexual imprinting is not constant across species. Cross-fostered great tits appeared to be imprinted on the blue tit host and failed to pair conspecifically. Cross-fostered blue tits, however, had high pairing success. Nevertheless, they were not unaffected by initial rearing conditions because in a few cases cross-fostered females nested with a male cross-fostered great tit. Pied flycatchers did not seem to be sexually imprinted on their tit hosts at all.

The present results support those of a previous cross-fostering experiment with great tits in the same study area, where we found that only a few great tits reared by blue tits paired with a conspecific bird as a yearling

(Slagsvold & Hansen 2001). The cross-fostered birds appeared to be strongly imprinted on the blue tit host, which seemed to explain the low pairing success. They associated with blue tits when foraging, their alarm calls resembled those of blue tits and, in the breeding season, they tried to form pairs with blue tits but failed (Slagsvold & Hansen 2001). In the present study, two cross-fostered females attempted nesting without any help from a social mate, as was observed for two other females in the earlier study, which is unusual for this strictly socially monogamous species (Björklund & Westman 1986; Slagsvold & Hansen 2001). Apparently, the low pairing success of cross-fostered great tits was not due to a lower quality of these birds than of controls as measured by initial rearing conditions, or body size and condition as adults. One explanation for their failure to mate is that they were so sexually imprinted on the host that they rejected pairing with conspecifics. Alternatively, they may

have been rejected by conspecifics because they failed to respond properly in sexual interactions. At present, it is not possible to separate between these alternatives and both may have been important. However, in both cases, sexual mis-imprinting seems to have been involved. Apparently, the mis-imprinting was stronger for cross-fostered great tits than for cross-fostered blue tits. This is supported by a separate experiment where a live bird was placed in a cage close to the nest box of breeding cross-fostered and control birds. Great tits reared by blue tits and blue tits reared by great tits showed similar and strong aggressive responses to intruders of the host species, indicating that both species were mis-imprinted. However, cross-fostered great tits failed to show such aggression to conspecific intruders, in contrast to cross-fostered blue tits and controls of both species. This indicates that great tits, but not blue tits, had lost the ability to respond properly to members of their own species because of the cross-fostering (B. T. Hansen and T. Slagsvold, unpublished data).

The most remarkable finding of the present study was the difference in the effect of cross-fostering between great tits and blue tits, as the two species are closely related and have similar breeding ecologies. Our study was done in the wild, in an environment natural to the birds where conspecifics of normal phenotype of both species were very common. Apparently, pairing did not seem to be more constrained in great tits than in blue tits because, in both species, almost every control bird was paired. The experiment was reciprocal and hence the difference in mate choice could not be explained by a difference in species relatedness. The two species breed at about the same time of the year and form mixed species flocks outside the breeding season. Great tits are socially dominant to blue tits, which may have reinforced the initial sexual imprinting of cross-fostered great tits. However, this explanation was ruled out by the fact that mate choice appeared to be unaffected when blue tits were cross-fostered to the smaller and subordinate coal tit. Note, however, that fewer coal tits were present in the study area, allowing fewer interactions with blue tits. Also the amount of parental care may influence sexual imprinting (ten Cate 1984). However, in great tits and blue tits, the presence of both parents is usually necessary for successful reproduction (Björklund & Westman 1986; Sasvari 1986) and so both sexes would usually be available as role models. Birds may not only use parents but also brood mates as role models in mate choice (ten Cate & Vos 1999; Slagsvold & Hansen 2001). However, it is difficult to see why the relative importance of parents and siblings as role models should differ between blue tits and great tits.

Alternatively, mating preferences may in general be less influenced by learning in blue tits than in great tits, for instance, if costs associated with species recognition errors are greater in blue tits because they may suffer severely if they engage in fights for food, roosts and nest sites with the larger and socially dominant great tit. Gene-related sexual traits may change more quickly under culturally inherited preferences (e.g. imprinting) than under genetically inherited preferences (Laland 1994). There seems to have been a greater species diversification in the *major*-line than in the *caeruleus*-line (Eck 1988) and, in the western palaeartic, more races have been described within the

great tit than the blue tit (Vaurie 1959; Cramp & Perrins 1993). Great tits thus seem to have experienced more recent changes in appearance than blue tits. It would be interesting to investigate whether this difference is a result of stronger sexual imprinting in great tits than in blue tits, or if the species diversification has caused a difference between the species in sensitivity to imprinting. Learning may enable an individual to adapt quickly to a changing environment, whereas an innate species recognition mechanism is more conservative (Immelmann 1972; Bolles & Beecher 1988; Witte *et al.* 2000).

Little social contact with the host species may explain why cross-fostered pied flycatchers were not sexually imprinted on the smaller blue tit or on the larger great tit. Pied flycatchers spend the winter in tropical Africa where the two species of tits are not found. Identification of a suitable mate may also depend less on an imprinting mechanism of learning in a solitarily living species than in a more social species because of little social contact with conspecific birds. In addition, pied flycatchers are not closely related to the tits, which may cause even further reduced sensitivity to imprinting if the sexual imprinting is template-guided, as suggested for the learning of songs in birds (Catchpole & Slater 1995). The fact that the flycatchers often are polygynous, with long distances in space between the two nests of a male (Lundberg & Alatalo 1992), may lead to little or no social contact between the chicks and an adult male, again selecting for sex recognition to be innate rather than learnt. Pied flycatchers may hybridize with the congeneric collared flycatcher *Ficedula albicollis* (Sætre *et al.* 1997; Veen *et al.* 2001) and this may be facilitated by cross-fostering (Löhrl 1955). Hybridization results in fitness losses (Sætre *et al.* 1997) and innate species recognition may help to avoid production of hybrids.

We report, to our knowledge, the first observations of pairings between blue tits and great tits. They occurred despite a large size difference between the two species. Only birds that had been cross-fostered were involved in heterospecific pairings. The pairings probably came about because the cross-fostered birds were ignored during mate choice by unmanipulated birds of the foster species (Slagsvold & Hansen 2001). In all cases of such pairings, a female of the smaller species (blue tit) paired with a male of the larger species (great tit). Such a size-based directional tendency in mixed pairs has been reported in some other vertebrates as well and may be caused by a general female preference for larger males (Grant & Grant 1997). However, this pattern does not seem to be universal in birds (Randers 2002) and abundances of the species and the operational sex ratios are probably more important (Grant & Grant 1997; Veen *et al.* 2001; Randers 2002).

Our study supports the view that, in case of heterospecific pairings, the resulting offspring need not be hybrids (Veen *et al.* 2001). Females may parasitize the resources provided by heterospecific males, including their territory, nest site and parental care. The study also shows that the two parties involved in mixed pairings need not be equally imprinted. Females of the least-imprinted species may more readily engage in such pairings than males because they have the opportunity to seek extra-pair copulations with a conspecific mate, thus duping the social mate into rearing heterospecific young. Females of the most-

imprinted species may let a male of the host species sire her offspring. However, hybrids may have low fitness, causing males of the least-imprinted species to avoid such pairings. Differences in the impact of learning may thus cause a skew in which sex from which species will form heterospecific pairs.

Birds may be very selective in mate choice and take subtle differences between potential mates into account (Andersson 1994; Jennions & Petrie 1997). In great tits, both male plumage colour (Norris 1990) and song quality (Baker *et al.* 1986) seem important for female choice. The fact that great tits apparently lack some ability to choose a conspecific mate when raised in the nest of another species indicates that mechanisms used to choose a mate of the correct species may be different from mechanisms used to choose between mates within a species. Why birds may differ in how early learning influences species recognition is unknown, but the findings should be taken into account in further studies of sexual selection, hybridization and brood parasitism (ten Cate & Vos 1999; Slagsvold & Hansen 2001). An avenue for further study would be to identify potential fitness benefits of learning from parents and siblings for optimal mating decisions. We also suggest that sexual imprinting be studied in more solitarily living species because most studies so far have dealt with social species (ten Cate & Vos 1999).

We thank R. Vestgård for permission to work in the area, Ø. Holen, G. F. Karlsen, T. E. Kjenn, J. E. Slagsvold and P. K. Slagsvold for assistance in the field and A. Cockburn, R. D. Magrath, T. Price and two anonymous referees for comments on the manuscript. The study was supported by a grant from the Norwegian Research Council and from the Nansen Foundation.

## REFERENCES

- Andersson, M. 1994 *Sexual selection*. Princeton University Press.
- Baker, M. C., Bjerke, T. K., Lampe, H. & Espmark, Y. 1986 Sexual response of female great tits to variation in size of males' song repertoires. *Am. Nat.* **128**, 491–498.
- Bakker, T. C. M. & Pomiankowski, A. 1995 The genetic basis of female mate preferences. *J. Evol. Biol.* **8**, 129–171.
- Bensch, S., Price, T. & Kohn, J. 1997 Isolation and characterization of microsatellite loci in a *Phylloscopus* warbler. *Mol. Ecol.* **6**, 91–92.
- Bischof, H. J. & Rollenhagen, A. 1999 Behavioural and neurophysiological aspects of sexual imprinting in zebra finches. *Behav. Brain Res.* **98**, 267–276.
- Björklund, M. & Westman, B. 1986 Adaptive advantages of monogamy in the great tit (*Parus major*): an experimental test of the polygyny threshold model. *Anim. Behav.* **34**, 1436–1440.
- Bolles, R. C. & Beecher, M. D. 1988 *Evolution and learning*. London: Lawrence Erlbaum.
- Catchpole, C. K. & Slater, P. J. B. 1995 *Bird song*. Cambridge University Press.
- Cramp, S. & Perrins, C. M. 1993 *Handbook of the birds of Europe, the Middle East and North Africa. The birds of the western palaearctic*. Oxford University Press.
- Eck, S. 1988 Gesichtspunkte zur Art-Systematik der Meisen (Paridae). *Zool. Abh. Staat. Mus. Tierkunde Dresden* **43**, 101–134.
- Grant, P. R. & Grant, B. R. 1997 Hybridization, sexual imprinting, and mate choice. *Am. Nat.* **149**, 1–18.
- Immelmann, K. 1972 Sexual and other long-term aspects of imprinting in birds and other species. *Adv. Study Behav.* **4**, 147–174.
- Immelmann, K., Pröve, R., Lassek, R. & Bischof, H.-J. 1991 Influence of adult courtship experience on the development of sexual preferences in zebra finch males. *Anim. Behav.* **42**, 83–89.
- Irwin, D. E. & Price, T. 1999 Sexual imprinting, learning and speciation. *Heredity* **82**, 347–354.
- Jennions, M. D. & Petrie, M. 1997 Variation in mate choice and mating preferences: a review of causes and consequences. *Biol. Rev.* **72**, 283–327.
- Kendrick, K. M., Hinton, M. R. & Atkins, K. 1998 Mothers determine sexual preferences. *Nature* **395**, 229–230.
- Kruijt, J. P. & Meeuwissen, G. B. 1991 Sexual preferences of male zebra finches: effects of early and adult experience. *Anim. Behav.* **42**, 91–102.
- Laland, K. N. 1994 On the evolutionary consequences of sexual imprinting. *Evolution* **48**, 477–489.
- Löhr, H. 1955 Beziehungen zwischen Halsband- und Trauerfliegenschnäpper (*Muscicapa albicollis* und *M. hypoleuca*) in demselben Bruchgebiet. In *Acta XI Congr. Int. Ornithol., Basel 1954*, pp. 202–203.
- Lundberg, A. & Alatalo, R. V. 1992 *The pied flycatcher*. London: Poyser.
- Norris, K. J. 1990 Female choice and the evolution of the conspicuous plumage coloration of monogamous great tits. *Behav. Ecol. Sociobiol.* **26**, 129–138.
- Oetting, S. & Bischof, H. J. 1996 Sexual imprinting in female zebra finches: changes in preferences as an effect of adult experience. *Behaviour* **133**, 387–397.
- Oetting, S., Pröve, E. & Bischof, H.-J. 1995 Sexual imprinting as a two-stage process: mechanisms of information storage and stabilization. *Anim. Behav.* **50**, 393–403.
- Otter, K. L. R., Michaud, D. & Boag, P. T. 1998 Do female black-capped chickadees prefer high ranking males as extra-pair partners? *Behav. Ecol. Sociobiol.* **43**, 25–36.
- Owens, I. P. F., Rowe, C. & Thomas, A. L. R. 1999 Sexual selection, speciation and imprinting: separating the sheep from the goats. *Trends Ecol. Evol.* **14**, 131–132.
- Penn, D. & Potts, W. 1998 MHC-disassortative mating preferences reversed by cross-fostering. *Proc. R. Soc. Lond. B* **265**, 1299–1306. (DOI 10.1098/rspb.1998.0433.)
- Perrins, C. M. 1979 *British tits*. London: Collins.
- Pomiankowski, A. & Möller, A. P. 1995 A resolution of the lek paradox. *Proc. R. Soc. Lond. B* **260**, 21–29.
- Price, T. 1998 Sexual selection and natural selection in bird speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 251–260. (DOI 10.1098/rstb.1998.0207.)
- Randers, C. 2002 Avian hybridization, mixed pairing and female choice. *Anim. Behav.* **63**, 103–119.
- Rice, W. R. 1989 Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
- Rollenhagen, A. & Bischof, H. J. 2000 Evidence for the involvement of two areas of the zebra finch forebrain in sexual imprinting. *Neurobiol. Learn. Memory* **73**, 101–113.
- Sætre, G.-P., Moum, T., Bures, S., Král, M., Adamjan, M. & Moreno, J. 1997 A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* **387**, 589–592.
- Sasvari, L. 1986 Reproductive effort of widowed birds. *J. Anim. Ecol.* **55**, 553–564.
- Slagsvold, T. 1998 On the origin and rarity of interspecific nest parasitism in birds. *Am. Nat.* **152**, 264–272.
- Slagsvold, T. & Hansen, B. T. 2001 Sexual imprinting and the origin of obligate brood parasitism in birds. *Am. Nat.* **158**, 354–367.
- Tanner, S. M. 1995 Microsatellite-DNA-fingerprinting in blue

- tits (*Parus caeruleus*) by the polymerase chain reaction. Diploma thesis, University of Bern, Germany.
- ten Cate, C. 1984 The influence of social relations on the development of species recognition in zebra finch males. *Behaviour* **91**, 263–285.
- ten Cate, C. & Vos, D. R. 1999 Sexual imprinting and evolutionary processes in birds: a reassessment. *Adv. Stud. Behav.* **28**, 1–31.
- Vaurie, C. 1959 *The birds of the palaearctic fauna. Passeriformes*. London: H. F. & G. Witherby.
- Veen, T., Borge, T., Griffith, S. C., Sætre, G.-P., Bures, S., Gustafsson, L. & Sheldon, B. C. 2001 Hybridization and adaptive mate choice in flycatchers. *Nature* **411**, 45–50.
- Witte, K., Hirschler, U. & Curio, E. 2000 Sexual imprinting on a novel adornment influences mate preferences in the Javanese mannikin *Lonchura leucogastroides*. *Ethology* **106**, 349–363.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.